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REVIEW

Nutrition and autoimmunity: a review

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INTRODUCTION

The profound effect on diet on the immune system was first recognized over 50 years ago with the observation of lowered resistance to infection and severe thymic atrophy in children suffering from protein-calorie malnutrition (PCM) (Jackson, 1925). It has now been established that both deficiencies and excesses in macro- and micronutrients can affect the immune response (reviewed by Beisel, 1982; Stinnett, 1983; Levy, 1984; Gershwin, Beach & Hurley, 1985). Thus, dietary manipulations have formed the basis of nutritional approaches to diseases of the immune system.

Autoimmune disorders are an important group of degenerative diseases in humans. Their study has been facilitated by the derivation of mouse strains which spontaneously develop manifestations of autoimmune rheumatic disease. Over the last decade, investigations using lupus-prone mouse strains such as the (NZB × NZW)F_1 (NZB/W) and the MRL/lpr/lpr (MRL/1) as well as other animals and human subjects have shown that variation in any of the major dietary components can bring about dramatic changes in the chronic, and often fatal, immunological perturbations seen in autoimmune diseases. In this review the influence of calorie restriction, fats, proteins, vitamins and minerals on these diseases is discussed. Emphasis is given to the potential use of these approaches for the prevention and treatment of human autoimmune disorders.

INFLUENCE OF CALORIE RESTRICTION ON AUTOIMMUNE DISEASE

Animal studies

The first study of calorie restriction in autoimmunity was conducted in conjunction with protein deprivation (Fernandes, Yunis & Good, 1976a) and used NZB/W and DBA/2f mice. The latter strain is immunologically normal but relatively short-lived. In both strains, survival was greatly enhanced by underfeeding, regardless of the protein content of the diet.

Subsequent studies on NZB/W hybrids examined the immunological alterations which occurred following calorie restriction. Underfed animals were found to be protected from immune nephritis and had a markedly prolonged life span (Friend *et al.*, 1978). The response of their spleen cells to T cell mitogens was increased: they had better preserved cytotoxic and plaque-forming cell responses to allogeneic cells, diminished production of anti-DNA autoantibodies and reduced deposition of gammaglobulins in capillaries of renal glomeruli (Fernandes *et al.*, 1978). The low-calorie diet also prevented the decline of splenic killer cell activity and T cell-dependent immune responses which occurs with age in high calorie-fed NZB/W mice. Moreover, restricting diet was effective in preserving the spleen production of and the thymus response to interleukin-2 in these same animals (Jung *et al.*, 1982). Such observations enlarged the immunological basis for nutritional modulation of the disease in lupus-prone mice.

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The New Zealand strains express large amounts of the xenotropic type C retrovirus, an endogenous virus that has been thought to contribute to the pathology of these mice (Levy, 1975, 1980). Further studies of calorie restriction showed that such diets were associated with reduced levels of circulating immune complexes, particularly those containing the retroviral envelope glycoprotein gp70, in the sera of NZB/W mice (Safai-Kutti *et al.*, 1980; Izui *et al.*, 1981). Calorie restriction also decreased C3 and Ig deposition in the kidneys. It had no effect, however, on endogenous production of infectious xenotropic retroviruses (Gardner *et al.*, 1977).

In studies on MRL/l mice, a doubling of their lifespan has been observed by reducing the total daily calorie intake by 50% (Fernandes & Good, 1979, 1984; Kubo, Day & Good, 1984). The major immunopathological parameters of these mice, including immune complex nephritis and T cell lymphoproliferation, were dramatically delayed by underfeeding, while their immunocompetence was generally preserved (Kubo, Day & Good, 1984). Histopathological abnormalities of the thymus, spleen, lymph nodes and kidneys of MRL/1 animals were also prevented by calorie restriction (Fernandes & Good, 1984). More relevant to clinical applications, the effects of dietary deprivation on longevity remained significant even when instituted later in life in both the NZB/W and MRl/1 strains (Friend *et al.*, 1978; Mark *et al.*, 1984). One important consideration from these studies is that reduced calorie intake delays the ageing process with which autoimmunity is associated (Good, 1981).

Human studies

There is an apparent contradiction between observations in mice and the effects of PCM on human systemic lupus erythematosus (SLE). Severely malnourished SLE patients failed to show any improvement in their anaemia, lymphopenia, hypergammaglobulinaemia, elevated autoantibody titres or arthralgia (Lockshin, 1980; Lom-Orta, Diaz-Jouanen & Alarcon-Segovia, 1980). Neither did PCM promote an increase in autoantibodies in lupus patients as compared to well-nourished control SLE patients (Youinou *et al.*, 1981).

These discrepancies probably result from the variations in experimental design of human and animal studies. Controlled animal studies do not reflect the clinical condition of severe PCM where multiple deficiencies, particularly of minerals, may accompany calorie deprivation. To date, very few experiments on reduced calorie consumption have been conducted on autoimmune patients. One recent study by Panush *et al.* (1983), which tested the effect of diet on rheumatoid arthritis (RA), included a regimen moderately restricted in calories (approx. 60% of the US RDA). Moreover, this experimental diet had a relatively high content of polyunsaturated fatty acids. The results showed no significant effect of the diet and are difficult to interpret. The protocol was not designed to test specific nutritional variables but examined instead a diet widely publicized in the lay press (Dong & Banks, 1973). The study thus could not include a true control group on a similar but non-restricted diet.

Another trial with RA patients showed that fasting was accompanied by reduction in joint pain, stiffness and medication requirements (Skoldstam, Larsson & Lindstrom, 1979). This study was also not well-controlled and lacked confirmatory results. Finally, symptoms of RA could not be attenuated by a lactovegetarian diet (Parke & Hughes, 1981).

In summary, it is not yet possible to conclude whether calorie restriction improves the pathology of human autoimmune diseases. Moreover, any positive impact of calorie restriction on autoimmunity is contingent on the feasibility of such intervention over prolonged periods of time.

EFFECTS OF FATS ON AUTOIMMUNITY

In the early studies on dietary fat and autoimmunity, very little attention was given to the balance of lipid components such as polyunsaturated and saturated fatty acids. Neither were the levels of calorie intake controlled. Fernandes *et al.* (1972) noted that two commercial diets differing in protein and fat content influenced the breeding behaviour, spontaneous disease and longevity of NZB mice. The high fat/low protein (high calorie) diet improved breeding performance, shortened the life span and fostered haemolytic anaemia in the animals, whereas the low fat/high protein (low calorie) diet reversed this trend and extended survival, particularly in males.

Table 1. Results of immune studies of 7-month-old female NZB/W mice receiving diets varying in fat content

Immune parameter	High saturated fat	High unsaturated fat	Low fat	Conventional rodent pellet
T cell mitogenesis	−	(−)	+	−
B cell mitogenesis	+	+	−	+
Bromelain plaque assay	N	++T helper	N	+T helper
Macrophage phagocytosis	−	−	N	(−)
NK cell activity	−	−	N	(−)
Interferon production	−	−	N	−
Response to IgG	−	+	−	+
i.p. SRBC IgM	NE	NE	NE	NE
Serum IgG, IgM	NE	NE	NE	NE
Autoantibody production	+NTA [IgM]	+dsDNA [IgG]	−	+dsDNA
Circulating IC	N	+	N	+
IC nephritis	+glom	+glom	(+)mesang	(+)mesang
Retroviral gp70	++	N	N	+
Neutralizing factor	+	+	N	N
Skin hypersensitivity to DNFB	NE	NE	NE	NE

glom = glomerular deposits; mesang = mesangial deposits; NK cell = natural killer cell; SRBC = sheep red blood cell; IC = immune complex; i.p. = intraperitoneal; N = normal; NE = no substantial effect; + = increased; − = decreased; ( ) = mild effect; DNFB = dinitrofluorobenzene.

Taken from studies by Levy *et al.* (1982), Morrow *et al.* (1985a), Yumura *et al.* (1985).

Since then, numerous studies have largely confirmed these observations, and investigators have concentrated on isolating the influence of fat on NZB/W mice by utilizing calorie-controlled diets differing only in qualitative and quantitative fat content. They examined the effects of saturated versus polyunsaturated fats on the progression of autoimmune disease. These studies showed that low fat diets significantly prolonged survival and delayed the onset of disease while high fat diets (regardless of the type of fatty acid content) enhanced immune complex nephritis and promoted the early death of NZB/W animals (Levy *et al.*, 1982; Fernandes *et al.*, 1983; Kelley & Izui, 1983; Morrow *et al.*, 1985; Yumura *et al.*, 1985). However, autoantibody production appeared to be selectively influenced by the degree of saturation of fat. Mice receiving a diet high in saturated fat exhibited enhanced titres of natural thymocytotoxic antibody, an IgM autoantibody, whereas mice given a diet high in unsaturated fat had increased levels of anti-DNA IgG autoantibodies (Levy *et al.*, 1982). Also, high fat diets appeared to have deleterious effects on natural killer cell activity and macrophage phagocytosis (Morrow *et al.*, 1985). A summary of the findings of the effects of various dietary fats on NZB/W mice is presented in Table 1.

In addition to serological alterations, histopathological examinations have shown specific effects of high fat diets on tissues of NZB/W mice. Yumura *et al.* (1985) showed that near-isocaloric, high saturated and unsaturated fat diets led to the deposition of immune complexes in the glomerular capillary walls of kidneys, whereas diets of lower fat content showed primarily mesangial deposits. This latter distribution is typical of kidneys of patients with mild SLE (WHO Classification of Renal Lupus, quoted by Barba *et al.*, 1983). Kelley & Izui (1983) showed that a very high concentration of saturated fat (50%) in the diet increased lipid deposition in the glomerulus, caused severe vascular injury, and accelerated the development of glomerulonephritis. Similarly, normocaloric high saturated fat (20%) diets have been associated with a high incidence of aortic and renal arteritis and atherosclerotic lesions in NZB/W mice while low fat/low calorie diets

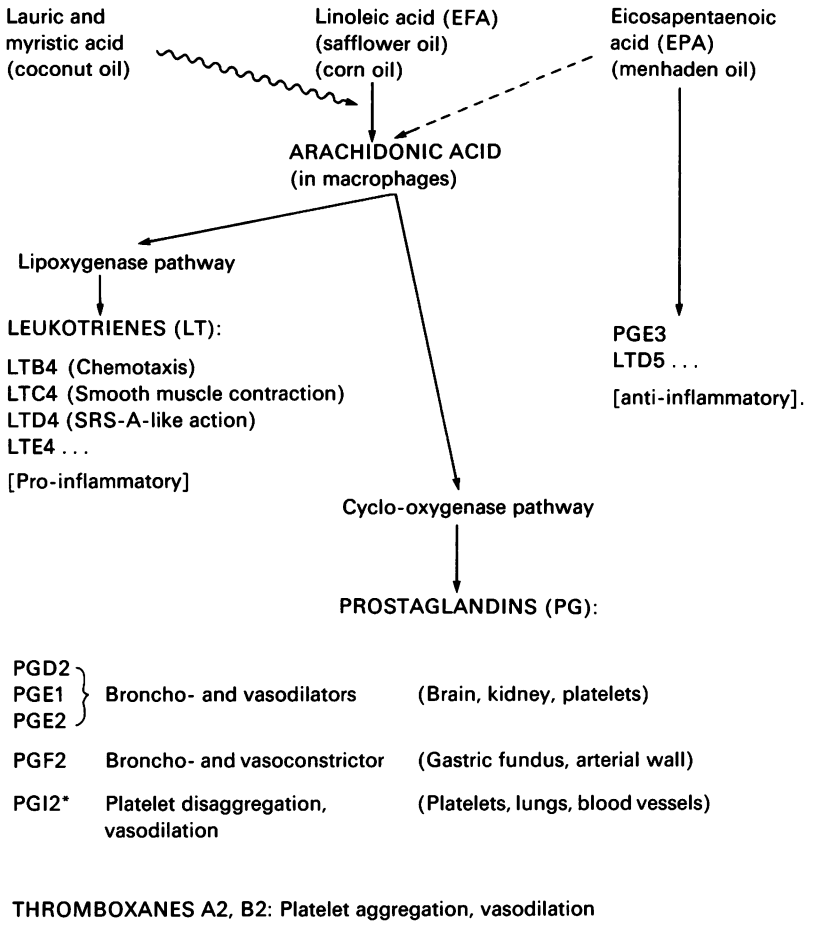

Fig. 1. Schematic diagram of prostaglandins (PG) and leukotrienses (LT) synthesis from polyunsaturated fatty acids (PUFA). The primary effects of PG products are shown. Linoleic acid, an essential fatty acid (EFA) found in most vegetable oils, particularly corn oil, is in mammals a major precursor for arachidonic acid (ArAc) which leads to the synthesis of LT through the lipoxygenase pathway, and to PG via the cyclo-oxygenase pathway. These two pathways appear to be interrelated. Eicosapentaenoic acid (EPA), a component of fish oils, is a minor precursor of ArAc and induces preferentially the formation of anti-inflammatory compounds, PGE_5 and LTD_3, which suppress the synthesis of inflammatory products of ArAc metabolism. Lauric and myristic acids are found in coconut oil and are antagonists of the transformation of linoleic acid into ArAc. (∿∿→) inhibitory effect; (– – →) poor precursor. *Also called prostacyclin.

prevented these pathological changes (Fernandes *et al.*, 1983). Finally, NZB/W mice maintained on a low fat diet experienced decreased manifestations of Sjögren's syndrome, a chronic autoimmune exocrinopathy characteristic of the immunopathology seen in these mice. The group fed low fat had decreased levels of lymphocytic infiltration in the lacrimal and parotid glands and preserved tear production as compared to their high fat fed counterparts (Swanson Morrow & Levy, 1986).

Other studies on NZB/W mice have shown that high saturated fat diets increase T helper cell hyperplasia and hyperfunction as well as prostaglandin (PG) levels in lymphoid tissues (Fernandes, 1983). These results supported the original proposition by Mertin & Hunt (1976) that the effect of fat on autoimmunity is modulated via the synthesis of PG and leukotrienes (LT). PG and LT form a family of potent inflammatory mediators with both immunoenhancing and suppressive effects.

Table 2. Fats, prostaglandins and autoimmune disease

Study	Animal model	Fat type and amount	Effects on disease	Production of PG or PG precursors	
Hurd *et al.* (1981)	NZB/W mouse	HSF 20%/EFA-d (coconut oil)	−	Lin Ac	−
		EFA 15% (safflower oil)	NE	Ar Ac	NR
Levy *et al.* (1982)	NZB/W mouse	HSF 18% (lard)	+		
		HUF 9% (corn oil)	+	PGE_2	NE
Prickett *et al.* (1981, 1983)	NZB/W mouse	EPA 5% (menhaden oil)	−	PGE_3	+
				LTD_5	
		HSF 33%/EPA-d (beef tallow)	+		NR
Kelley *et al.* (1985)	MRL/1 mouse	EPA 20% (menhaden oil)	−	PGE_3	+
				PGE,PGI_2	−
		EFA 20% (safflower oil)	+	PGE_3	−
Leslie *et al.* (1985)	B10 mice (Type II collagen arthritis)	EPA 5% (menhaden oil)	−	PGE_2	−
				PGI_2	
		EFA 5% (corn oil)	+	PGE_2	+
				PGI_2	

HSF = high saturated fat; HUF = high unsaturated fat; EFA(−d) = essential fatty acid (−deficient); EPA-(−d) = eicosapentaenoic acid (−deficient); PGE, PGI = prostaglandin E, I; LTD = leukotriene D; TXB = thromboxane B; + = enhanced; − = reduced; NR = not reported; NE = no effect; Lin Ac = linoleic acid; Ar Ac = arachidonic acid.

They are physiologically active at very low concentration, but the precise balance involved in normal immunity is not yet known. Thus, any effect on either arm of the PG/LT system may give a different outcome. This fact is reflected by the diversity of responses obtained by investigators who examined the PG/LT hypothesis.

Fatty acids, prostaglandins and leukotrienes

The major biochemical precursor of PG is the polyunsaturated fatty acid (PUFA) arachidonic acid (ArAc) (Fig. 1); the essential fatty acid (EFA) linoleic acid is in mammals a necessary precursor for the biosynthesis of ArAc (Lehninger, 1979).

Animal studies. Initially, diets rich in linoleic acid were assumed to foster the production of immunostimulatory PG in lupus-prone mice and thereby increase the inflammatory process responsible for the progression of the disease. This hypothesis has since gathered conflicting evidence (summarized in Table 2). While a diet rich in saturated fat, but deficient in EFA, markedly increased survival and reduced all manifestations of the disease in NZB/W mice, a diet of 15% safflower oil (this oil contains 78% linoleic acid) did not exacerbate the disease as expected (Hurd & Gilliam, 1981; Hurd *et al.*, 1981). The investigators proposed that, in addition to the PG effect, EFA-deficient diets might affect the hormonal balance of the animals, which is known to influence the course and severity of SLE both in NZB/W mice and in humans. Nevertheless, other studies (Levy *et al.*, 1982) showed that PGE_2 production by mononuclear cells did not differ in NZB/W

mice fed diets containing either high saturated (18% lard), high unsaturated (9% corn oil) or low (1·2% corn oil) fat, although the disease was markedly accelerated and the mitogenic response of T cells was much reduced in the high fat fed animals. The high unsaturated fat diet was also associated with a high mortality rate and increased levels of anti-dsDNA autoantibodies in MRL/1 mice, whereas animals fed the high saturated fat diet were found to have macrophages with the lowest level of phagocytic activity (W. J. W. Morrow *et al.*, unpublished). In contrast, the survival of low fat fed MRL/1 mice was greatly prolonged and their immune function better preserved, particularly interleukin-2 production.

Eicosapentaenoic acid (EPA) is a minor precursor of ArAc and can be broken down into anti-inflammatory forms of PG and LT (Fig. 1). For this reason, fish oil, which is rich in EPA, has been used in experimental diets in animals and humans. NZB/W mice placed on an EPA-rich fish oil diet were protected from autoimmune nephritis and this effect was still observed when the diets were instituted later in life (5 months of age) (Prickett, Robinson & Steinberg, 1981). Such results could not be attributed to levels of anti-DNA autoantibodies that were found to be unchanged until late in the life of the animals (12 months of age). Rather it appeared that the EPA-rich diet favoured the formation of the immunoinhibitory substances PGE_3 and LTD_5 (Prickett, Robinson & Steinberg 1983).

A fish oil diet was also able to suppress the autoimmune sequellae in MRL/1 mice (Kelley *et al.*, 1985). When compared to a safflower oil-fed group, the fish oil-fed mice had decreased T cell lymphoproliferation and reduced expression of Ia antigen on peritoneal macrophages. The diet was associated with low PGE, thromboxane B2 and prostacyclin levels in the lungs and kidneys, and increased renal PGE_3 levels. It also prevented the formation of circulating retroviral gp70 immune complexes and delayed the development of immune nephritis. Consistent with these findings are those of Leslie *et al.* (1985) who found that fish oil protected mice from collagen-induced autoimmune arthritis, lowering their PG levels and altering their macrophages' fatty acid content.

In summary (Table 2), whereas the presence of linoleic acid did not always enhance the autoimmune symptomatology in the different animals tested, the absence of ArAc precursors in the diet as well as the supplementation with EPA resulted in significant improvement of the animals' survival and pathology. This result can be in part explained by the fact that EPA preferentially induces the production of immunoinhibitory PG or prevents the formation of inflammatory PG or LT (Needleman *et al.*, 1979; Hammarstrom, 1980). In any event, the balance and interplay of the different PG and LT on the immune system must be considered when evaluating the influence of various fat diets on autoimmune diseases.

Human studies. A clinical trial testing diets rich in EPA in RA patients has recently been reported (Kremer *et al.*, 1985). In a double-blind study, patients were given diets either high in PUFA with a fish oil supplement or high in saturated fat with a placebo supplement. After 12 weeks, patients on the fish oil diet had significantly reduced morning stiffness, a decreased number of tender joints and increased grip strength in comparison with the control group. There were no remarkable differences observed in erythocyte sedimentation rates or titres of rheumatoid factor. When both groups were permitted to return to their regular diets, differences in clinical signs were no longer detected. This result was explained by the experimental group returning to saturated fats and 'allergenic' foods such as dairy products and the control group adding PUFA back to their diet (Birtwistle & McEwen, 1985). Nevertheless, despite the limitations of this study (the relatively short time the patients were studied and the lack of immunological tests), its outcome is encouraging; it suggests that dietary manipulation might be a feasible form of therapy for autoimmunity in humans.

The study by Panush *et al.* (1983) on RA patients, mentioned previously also tested a diet relatively rich in EPA since its sole source of animal protein was fish. Although the results were not significant clinical improvements were observed on two of the test patients. Along with the findings of Kremer *et al.* these observations suggest that further experiments with fish oil in human autoimmune disorders may prove beneficial.

Lipoproteins

The mechanisms by which fats modulate immune responses may also involve production of immunoregulatory lipoproteins (LP). For example, diets high in fat have been associated in mice

with greatly increased levels of a serum neutralizing factor (NF), an LP that specifically neutralizes the aforementioned endogenous xenotropic type C retrovirus found in all mice, but expressed in very high titres in New Zealand strains (Levy, 1975, 1977, 1980; Levy *et al.*, 1975). This observation has led to the hypothesis that some interaction between NF and xenotropic retroviral envelope glycoproteins (e.g. gp70) on lymphocyte surfaces might affect the function of these cells and thereby contribute to the pathology seen in the mice (Levy, 1975, 1977, 1980).

In conclusion, low-fat, near-isocaloric diets (< 2·5% fat) given to lupus-prone mice significantly and consistently delayed the onset and reduced the severity of autoimmune manifestations, improved immunological responses and prolonged survival. Although the mechanisms remain unknown, the connection between fatty acids and PG/LT production can at least partially acount for this strong modulation of autoimmunity by dietary fat. Other mechanisms include a possible regulatory effect of lipoproteins on lymphocytes (Curtiss & Edington, 1976) and/or a direct action of lipids and fatty acids on the composition and fluidity of immune cell membranes (Schroit & Gallily, 1979; Gurr, 1983).

PROTEINS, AMINO ACIDS AND AUTOIMMUNE MECHANISMS

Animal studies

When NZB mice were submitted to chronic protein restriction (6% casein) with no difference in calorie intake, the onset of their disease was delayed but no increase in lifespan was observed (Fernandes, Yunis & Good, 1976b). Thymic involution, splenomegaly, decreased antibody production and cell-mediated cytotoxicity against allogeneic cells observed in the control group (fed 22% casein) were prevented by the protein restriction in test animals. This dissociation between surivival rate and immunological parameters could be a result of the moderate degree of protein restriction or still reflect disproportionate susceptibility of cell populations (e.g. suppressor cells) involved in the disease. This last consideration could explain why moderate protein restriction reduced antibody production in normal mice (e.g. Cooper, Good & Mariani, 1974) while it restored this parameter in autoimmune mice. When protein and calorie restrictions were combined, protein restriction (6%) alone was less effective than caloric deprivation in prolonging the survival of NZB/W mice (Fernandes *et al.*, 1978). However, DBA/2f mice, the normal short-lived strain used as a control, had a better survival rate when they were restricted in protein only. NZB/W animals were more affected in their survival by protein restriction than NZB mice. This observation most probably reflects the differences in pathology between the two mouse strains. Since NZB/W mice develop autoimmune disease faster than NZB mice, they can be expected to respond more acutely to any factor likely to influence their immunopathology.

Selective amino acid restriction also influences the course and development of autoimmune pathology in lupus-prone mice. A diet low in phenylalanine and tyrosine prolonged survival more than twofold and had a profound inhibitory effect on the nephropathy of NZB/W mice (Dubois & Strain, 1973; Gardner *et al.*, 1977). In particular, glomerulonephritis and immune complex deposition were significantly reduced in kidneys of the amino-restricted mice. However, this diet, like low-calorie diets, had no influence on the expression of the endogenous type C xenotropic virus (Gardner *et al.*, 1977).

More recently, striking overall improvement of murine lupus was obtained by the use of synthetic amino acid preparations. When female NZB/W were given a fully synthetic amino acid diet otherwise similar in composition to a standard pellet diet, their survival was markedly prolonged and an equally dramatic fall in levels of antinuclear antibodies, circulating immune complexes and proteinuria was observed (Batsford *et al.*, 1984). These results confirmed a preliminary report by the same investigators, in which the synthetic amino acid diet was either used alone or supplemented with histidine and/or zinc (Schwerdtfeger *et al.*, 1980). In that study, histidine and zinc tended to further ameliorate the effects of the synthetic amino acid diet. The investigators proposed that the synthetic amino acid diet could be devoid of some proteins that have been shown to act as antigens or adjuvants which activate B cell clones (Primi *et al.*, 1977; Rothberg, Kraft & Michalek, 1973).

Similarly, a diet in which natural proteins were replaced with synthetic L-amino acids had a protective effect on Wistar BB rats, a strain that spontaneously develops insulin-dependent, autoimmune diabetes (Nakhooda *et al.*, 1972). When BB rats were placed on such a diet, the incidence of the disease decreased from 50% to 15% (Elliot & Martin, 1984), suggesting that antigens associated with dietary proteins may be involved in the pathology of this disease.

Mutagens

The protective effect of synthetic amino acid diets points to a possible relationship between food processing and autoimmunity. Food processing, especially at high temperature, generates molecules such as free radicals, Maillard compounds (the products of reaction between amino acids and sugars), and the burnt or browned material of cooked food proteins. These products are found in substantial quantities in the diet (Sugimura & Nagao, 1982; Bjeldanes *et al.*, 1982; Ames, 1983). Although their physico-chemical, mutagenic and nutritional characteristics have been extensively studied (Eriksson, 1982; Vernin, Metzger & Obretenov, 1983), very little is known of their effects on the immune system. Yet, purified allergens have been shown to be similar in structure to Maillard compounds (Berrens, 1971), and free radicals are known to damage gammaglobulins *in vitro* (Dormandy, 1983a, b). Furthermore, diets rich in mutagens (smoked-cured meat) have been associated with an increased frequency of type I diabetes, a disease of autoimmune aetiology at least in part. When either normal mice (Helgason *et al.*, 1982) or humans (Helgason & Jonasson, 1981) have a high consumption of smoked-cured meat around the time of conception, diabetes occurs with higher frequency in the offspring.

Food antigens

Food antigens, particularly proteins, are capable of inducing crossreactive autoimmunity in much the same way as do infectious agents and pharmacological substances (Cooke, Lydyard & Roitt, 1983, 1984). Indeed, bacterial, viral or drug antigens, even without adjuvants, as well as the antibodies they elicit, can cross-react in several ways with self-tissues (auto-antigens) or normal autoantibodies and induce pathological autoantibody production leading to tissue damage and disease (Izui *et al.*, 1979; Plotz, 1983; Reidenberg *et al.*, 1983; Cooke, Lydyard & Roitt 1983, 1984). Thus, antibodies to the cereal proteins gliadin and gluten, and to reticulin, a tissue glycoprotein made of collagen and fibronectin, have been associated with coeliac disease, RA (Williamson, Housley & McCormick, 1967), dermatitis herpetiformis (Lane, Huf & Weston, 1982), and, more recently, Sjögren's syndrome (Teppo & Maury, 1984).

Recent studies have demonstrated that a diet of cows' milk can elicit a RA-like syndrome in Old English rabbits. The rabbits developed inflammed synovia as well as antibodies to milk proteins including Clq, conglutinin, beta-lactoglobin and IgG (Coombs & Oldham, 1981; Welsh *et al.*, 1985; Hanglow *et al.*, 1985). The authors hypothesize that this pathology is due to the formation and deposition of immune complexes but do not exclude the fact that dietary change may alter the gut flora in the rabbits and allow the proliferation of arthritogenic bacteria. This latter phenomenon has been reported in piglets fed fish meal, which later developed synovitis and glomerulonephritis accompanied by an increase in intestinal *Clostridium perfringens* (Mansson *et al.*, 1971). Whether such observations reflect a primary phenomenon underlying the disease or a consequence of the increased permeability of an inflamed intestinal mucosa to food antigens (resulting from, e.g. vitamin A deficiency) is not definitely established.

Human and animal studies clearly suggest that food antigens are also able to directly induce autoimmune disease. For instance, the amino-acid L-canavanine, found in large amounts of alfalfa sprouts or seeds, elicited the formation of an antibody cross-reacting with DNA (Bardana *et al.*, 1983), and induced an SLE-like syndrome in macaque monkeys supplemented with these foods (Bardana *et al.*, 1982; Malinow *et al.*, 1982). This disease was accompanied by elevated anti-nuclear antibodies and anti-dsDNA antibody titres, reduced C3 levels, and anaemia. The aetiology was confirmed when the disease reappeared in convalescent animals fed a diet supplemented with 1% L-canavanine alone. This observation was also supported by a report on two lupus patients who suffered disease exacerbation after ingestion of alfalfa tablets (Roberts & Hayashi, 1983).

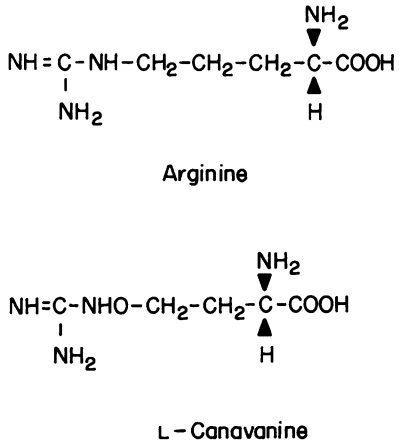

Fig. 2. Structural resemblance between the amino acids L-canavanine and arginine.

L-canavanine structurally resembles arginine (Fig. 2), a major amino acid constituent of histones (nuclear proteins), and may be incorporated in histones *in lieu* of arginine. This transposition might in turn affect genetic transcription and lead to synthesis of abnormal proteins such as autoantibodies (Malinow *et al.*, 1982). Various non-protein amino acids that are similar to mammalian amino acids occur in higher plants (Robinson, 1975). Whether their dietary absorption would have the same autoimmune-inducing effects as L-canavanine and its derivatives remains to be explored.

In view of these results, additional experiments addressing the relevance of food antigens to self-recognition mechanisms are required. It is not known, for instance, how lymphocytes learn to tolerate food immunogens that enter the system and whether these antigens bind to self-tissues (Unsworth *et al.*, 1981) and 'mark' cell membranes, as do infectious agents or drugs. Should food antigens be involved in such mechanisms, their relative impact on immune function and disease could be greater than any other environmental factor, given the amount and frequency of food absorption.

MINERALS AND AUTOIMMUNITY

Many trace minerals have been recognized as essential nutrients for normal growth, development and physiological function in humans. Data are accumulating on their influence on the immune system (for review see Beisel, 1982; Chandra, 1983; Levy, 1984; Gershwin *et al.*, 1985). However, apart from a recent study showing that iodine modulates autoimmune thyroiditis in genetically susceptible chickens (Bagchi *et al.*, 1985), only zinc has been extensively studied in relation to autoimmunity.

Zinc

When young NZB mice were submitted to diets with marginal (9 ppm), moderate (5 ppm) and severe (2·5 ppm) zinc deficiency, the more deprived groups (5 ppm and 2·5 ppm) showed delayed onset of haemolytic anaemia, reduced levels of anti-erythrocytes autoantibodies and serum immunoglobulins, and increased survival (Beach, Gershwin & Hurley, 1981). In addition, zinc deprivation altered the progression of the disease more significantly than calorie restriction alone was able to achieve in the control groups. These controls were pair-fed a diet not deficient in zinc but matching the caloric intake of the test groups, in order to account for the loss of appetite induced by zinc deficiency. When instituted later in life, zinc restriction also showed the haemolytic anaemia but this effect was most likely due to the inanition induced by zinc deprivation.

A similar study with female NZB/W mice (Beach, Gershwin & Hurley, 1982a) gave the same results. The moderately and severely restricted groups experienced a prolonged survival due to delayed renal pathology, reduced anti-dsDNA antibody titres and decreased proteinurea.

When low zinc diets were given early in life, the effects were more substantial than in the group restricted at a later age. Zinc deprivation also exerted a more significant influence than the accompanying calorie restriction. Among the seroimmunological parameters tested, levels of IgG2b and IgA were reduced. This finding suggests that zinc deficiency could have altered the production of autoantibodies in the deprived animals, since its availability affects T cell blastogenic transformation (Berger & Skinner, 1974), and polyclonal B cell activation by mitogens *in vitro* (Cunningham-Rundles, Dupont & Good, 1980) as well as the mobility and distribution of B cell surface immunoglobulins (Maro & Bornens, 1979). Zinc deprivation has also been shown to stimulate the reticuloendothelial system (Chvapil, 1976) which is responsible for immune complex catabolism. Thus, the persistence of immune complexes in the mice may have been affected as well by zinc deficiency. However, histoimmunopathological parameters were not analysed in these experiments and these hypotheses need further investigation.

The above results on zinc deprivation were confirmed with MRL/l mice (Beach, Gershwin & Hurley, 1982b). A lack of this mineral, independent of caloric deprivation, was able to prolong significantly the life span of the animals as well as delay the appearance and severity of autoimmune manifestations including arthritis, dermal necrotic lesions, glomerulonephritis and autoantibodies. Older zinc-deprived animals did not survive longer than their isocalorically pair-fed controls. Younger animals were more responsive to zinc deprivation than their older counterparts, probably because of the importance of zinc for normal thymus function before sexual maturation (Bach, 1981). Yet, zinc deprivation, whether instituted in young or older MRL/l mice, significantly reduced the massive lymphoproliferation that is characteristic of the pathology of these mice (Beach, Gershwin & Hurley, 1982b).

Although of fundamental interest, zinc deprivation is likely to be of limited clinical application for the management of human autoimmune disorders such as SLE and RA. These diseases affect mostly adult individuals (Dubois, 1974), whose immune system might not be very responsive to zinc deficiency. Moreover, a lasting zinc restriction is not without risks as it may lead to acrodermatitis enteropathica, a human disease caused by chronic zinc malabsorption.

VITAMINS IN AUTOIMMUNE PROCESSES

The importance of vitamins in immune responses has been recognized (reviewed by Beisel, 1982; Gerschwin *et al.*, 1985). However, as with minerals, there are to date few available studies on the influence of vitamins on autoimmune diseases.

Vitamin A

Recently, Gershwin *et al.* (1984) investigated the possibility that the delay in autoantibody production induced by zinc deprivation in NZB mice is mediated in part by the accompanying decrease of vitamin A levels. At 6 months of age, test animals were placed on a normal-zinc, vitamin A-deficient diet and the controls were given *ad libitum*, or pair-fed, the same diet with normal amounts of vitamin A. Contrary to expectations, the vitamin A-deficient animals were found to have a more severe hypergammaglobulinaemia and an earlier onset of natural thymocytotoxic antibodies as well as IgM anti-erythrocyte autoantibodies than the control groups. It is thus unlikely that the effects of zinc deficiency on autoimmunity are mediated by secondary vitamin A deficiency (Suskind, 1981). This experiment also suggests that vitamin A deficiency itself could be another independent variable entering the complex processes by which nutrition can influence the course of autoimmune disease.

Vitamin E

Vitamin E has been found to preserve immune function in normal and lupus-prone ageing mice. NZB mice fed a vitamin E-enriched diet, or supplemented with a synthetic vitamin E-like

antioxidant survive significantly longer than controls and show reduced signs of kidney damage (Harman, 1980). These results support the hypothesis that the increase of autoimmunity with age parallels a higher rate of oxidative processes induced by free radicals (Dormandy, 1983a, b).

In humans, clinical studies have found that large daily supplementation of vitamin E preparations (800–1600 iu) induced remissions in patients suffering from a variety of autoimmune diseases including SLE (Mihan & Ayres, 1979) as well as scleroderma and polymyositis among others (Ayres & Mihan, 1978). These observations, however, were weakened by the fact that controls were not included.

Finally, in view of the relationship between PG and autoimmune processes, it is of interest to note that vitamin E can regulate the immune response through changes in PG levels as demonstrated by the fact that PG synthesis is diminished in bursa and spleen cells of chicks supplemented with dietary vitamin E (Likoff *et al.*, 1978).

Biotin

Biotin, a vitamin playing an important role in fatty acid synthesis and glucose formation, is required for normal antibody production and T cell proliferation both in rats and humans (Kumar & Axelrod, 1978; Cowan *et al.*, 1979). The effect of biotin deficiency on autoimmunity has been studied in relation to experimental allergic encephalomyelitis (EAE), an autoimmune disease experimentally induced upon immunization with guinea pig myelin basic protein (MBP) (Rabin, 1983). In this study, rats were fed a diet containing 20% egg white (ovalbumin) which binds to biotin and reduces its dietary availability. When these biotin-deficient rats were immunized with MBP, they failed to develop the characteristic clinical profile of EAE with progressive hind leg paralysis followed by remission seen in the controls (Linthicum, Mackay & Carnegie, 1979). Moreover, the transfer of lymphocytes of MBP-immunized, biotin-deficient rats into either test or control rats did not produce symptoms of paralysis as did lymphocytes from the control animals (Rabin, 1983). This observation confirmed an early report where EAE was similarly prevented in mice receiving a synthetic diet deficient in biotin (Schneider, Lee & Olitsky, 1957). Overall, these results suggest that biotin could regulate pathological lymphoproliferation in this model of autoimmunity. The potential effect of biotin on lupus-prone mice or human autoimmunity remains to be explored.

Vitamin C

A similar study was conducted on EAE-affected guinea pigs rendered deficient in vitamin C and the same results were obtained (Mueller *et al.*, 1962). Vitamin C seems particularly important for phagocytic cell viability and function, while it exerts a moderate effect on T cell populations (Anderson *et al.*, 1980). Thus, decreased symptoms of EAE seen in vitamin C-deficient guinea pigs could be ascribed to reduction of inflammation through impaired chemotaxis and lymphoproliferation.

Ascorbic acid is known to act as an anti-oxidant in the presence of free radicals (Leibovitz & Siegel, 1981). As such, it is likely to reduce the degenerative, and thus ageing, processes associated with autoimmunity. This vitamin also appears to be required for the normal production of thymic hormone (Bach, 1981). Therefore, the effect of this relatively non-toxic vitamin on autoimmunity should be further tested in animal experiments or clinical trials.

Vitamin B_{12}

Vitamin B_{12} is a coenzyme necessary for DNA synthesis. It is therefore essential for all cell reproduction, including lymphopoiesis. Early clinical trials investigating the effect of vitamin B_{12} on autoimmunity failed to show improvement of the disease in lupus patients (Goldblatt, 1951) and gave inconsistent results with RA patients (Rosenberg, 1954).

DISCUSSION

Studies on animal models have demonstrated that, without any doubt, nutrition is able to delay, prevent, and even reverse the expression of genetically determined autoimmune defects. This

finding opens a whole new area of research and experimentation towards the understanding and treatment of autoimmune diseases.

The status of research on nutrition and autoimmunity is in its infancy. Dietary intervention is a complex procedure and the problems in designing and conducting carefully controlled experiments with different nutrients, even in a laboratory environment, should not be underestimated. Most studies described to date have attempted to isolate a single nutrient and study its impact, most often in autoimmune-prone mice. Although of fundamental value, this approach falls short of reproducing the highly complex conditions of human nutrition, regardless of its feasibility in the medico-social context.

Despite these limitations however, recent evidence suggests that nutrition can also modulate the expression of autoimmunity in humans. In this perspective, diet and autoimmunity should be investigated from the point of view of therapeutic usefulness and more clinical trials should be initiated in order to answer specific questions on the safety and feasibility of their administration. Thus far, there has been a too limited number of studies in this direction and the few available often lacked sound design. In such interventions, different outcomes may occur due to each individual's pathological, genetic, nutritional and psycho-physiological status. Accordingly, both experimental and individual variables should be considered in the dietary management of autoimmune conditions. Clinically, it is conceivable to adopt a trial-and-error therapeutic approach based on available experimental data. Thus, a patient's diet could be modified according to the prevalent scientific evidence, but only the rate of success would determine which dietary changes should or should not be eventually adopted in each case. This approach might represent a socially more acceptable type of intervention and thereby reduce the lack of compliance, a major problem in such attempts.

As mentioned above, the 'single nutrient' approach has so far prevailed in experiments on diet and autoimmunity. Recent advances in nutritional physiology suggest that this approach may be simplistic and should be complemented by a food-oriented intervention. For example, it has been a long-established dogma in nutrition that the rate of absorption of carbohydrates (CHO) by the gut is solely a function of their chemical structure. Particular types of CHO, however, have been found to be absorbed in a totally unpredictable manner, depending on the type and physical form of the food in which they are ingested (Crapo & Olefsky, 1983). In other words, from a physiological point of view, there exists as many types of CHO as there are different CHO-containing foods. This observation implies that nutrients should no longer be considered separately from their food environment when accounting for their effects *in vivo*. What is true for CHO absorption may well apply to other nutrients, possibly at various metabolic levels. Experiments comparing the influence on autoimmune mechanisms of specific nutrients when present in different foods would be important for clinical applications.

Finally, the mechanisms presiding over immune responsiveness to food antigens should be examined. The possibility that food compounds initiate autoimmune reactions by inducing the formation of self-reactive idiotypes deserves particular attention. By modulating the balance between tolerance and immune responses to food antigens, nutrition may contribute further or to help reduce the physiopathology of autoimmunity. In conclusion, approaches at diet therapy in human autoimmune disease should be attempted with particular attention to calorie content, the amount and type of fat, and food antigens.

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